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TETRAZOLIUM VIABILITY, GERMINABILITY, AND SEEDLING GROWTH OF OLD SEEDS OF 36 MOUNTAIN RANGE PLANTS

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ABSTRACT

Seeds of 36 species of mountain range plants (6 trees, 12 grasses, 18 forbs) collected during 1928-31 at the Great Basin Experiment Station in Ephraim Canyon, Utah, were tested for viability and germinability in 1972. Twenty-nine species gave negative reactions in the tetrazolium seed viability test. The remaining species (3 grasses, 4 forbs), when tested for germination at each of three temperature alternations in the 25° to 5°C range, and at a stratification temperature, yielded low to intermediate percentages at two or more of the test temperatures. Survival of seedlings transplanted to the greenhouse was generally good, but only Agoseris glauca *completed the growth cycle to seed-set. Progeny (F_1) seeds of* A. glauca *failed to germinate unless the embryo was isolated by removal of the external tissues.*

Numerous investigations have been carried out on the viability in old seeds, as reviewed by Crocker (1945) and Quick (1961). Seeds of 8 of 20 common weed species buried in soil by Beal in 1879 retained germinability 40 years later (Kivilaan and Bandurski 1973). Tiedemann and Pond (1967) obtained germination in 4 of 20 species of grasses after 20 years storage under uncontrolled conditions. Hull (1973) examined seeds of 60 rangeland species that had been stored in an unheated shed for varying period of 14 to 41 years. All grass species lost germinability after 27 years; shrubs after 20 years and, among forbs, only *Erodium cicutarium* attained appreciable longevity (37 years).

Generalizations regarding the basis for the longevity of seeds are few (Crocker 1945, Quick 1961, Abdul-Baki and Anderson 1972). Certain anatomical and morphological characteristics of the seed, such as an indurated testa or pericarp, tend to promote longevity by reducing harmful fluctuations in the water content of the tissues. Natural storage conditions (in soil in the field) or proper laboratory treatments (drying and storing the seeds in sealed containers under refrigeration) also promote longevity. Loss of germinability with age probably results from denaturation of protein and nucleic acids of the genetic material. Aged seeds and derived seedlings characteristically exhibit reduced germination compared to new seed, changes in environmental requirements for germination, and slow, sometimes abnormal seedling growth.

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MATERIALS AND METHODS

Seeds that had been collected on high elevation range in Ephraim Canyon, Utah, between 1928 and 1931 for a study involving the effects of alternate wetting and drying on germination (Griswald 1936) were tested for germinability in 1972. The 41 to 44 year-old-seeds of 36 species had been stored in paper packets in a building of the Great Basin Experiment Station under uncontrolled conditions. Included were 6 species of trees, 12 grasses, and 18 forbs.

A preliminary screening for tetrazolium viability (Moore 1969; Abdul-Baki and Anderson 1972) was made on 50 seeds of each species. The seeds were imbibed on water-saturated filter paper in petri dishes for 6 hours before being bisected longitudinally. The halves were immersed in 25 ml portions of 1 percent aqueous 2, 3, 5-triphenyl-2H-tetrazolium chloride for 6 h in the laboratory before color evaluations were made. Seeds of those species that gave a positive color reaction in any percentage were tested for germination.

Germination tests were made with filter paper saturated with distilled water in 9-cm petri dishes (Florez and McDonough, 1974). Three daily temperature alternations (25°/15°C, 20°/10°C, 15°/5°C) were used. Seeds were exposed to the higher temperature for 8 h and then to the lower for 16 h. Eight h photoperiods coincided with the higher temperature of the alternation. Four replications of each temperature treatment were made with 25 or 50 seeds per dish. Tests were run for 30 days with examinations for germination (visible root protrusion) every third day. Other sets of 25 or 50 seeds per dish with four replications were stratified for 120 days at 2° ± 1°C before examination for germination. Ungerminated seeds were then transferred to 30 days of temperature alternation (20°/10°C) and examinations.

New seeds of several of the species were collected in 1972 on the same old collection sites in Ephraim Canyon and tested for germination under the same conditions. Other germinability comparisons were based on results published by Griswald (1936) and on collections made in the late 1960's on high elevation range on the Dixie and Uinta National Forests in Utah (McDonough 1969; 1970).

Plant hormones or other biochemicals known to promote germination in old and dormant seeds--biochemicals that might substitute for natural constituents that had deteriorated or for which the synthesizing systems had deteriorated--were applied to old seeds showing some germinability in an attempt to increase the germination percentages. The biochemicals used were ethylene (Takayanagi and Harrington 1971), gibberellin A_3, A_7, kinetin (Wareing and Saunders 1971), and ribonulease (Wurzburger and Leshem 1971). Concentrations and methods of application were taken from the literature. Procedures and incubation temperatures were the same as those in the principal experiment.

Seedlings derived from germination of old seeds were planted in mountain topsoil, two seedlings per 20 l container, to determine survival and growth over a 4-month period under greenhouse conditions. Surviving plants that showed arrested development were then placed in a cold room at 2° ± 1°C for 4 months before return to the greenhouse for an additional 4 months of observation.

In another experiment, embryos of progeny (F_1) seeds of *Agoseris glauca* were isolated from the external tissues (endosperm and pericarp) by imbibing for 8 h, slitting the external tissues near the root end with a dissecting needle, and extruding the embryo by pushing against the cotyledonary end.

RESULTS AND DISCUSSION

Of the 36 species, 29 were negative in the tetrazolium test:

TREES

Abies concolor (Gord. & Glend.) Lindl.
Acer glabrum Torr.
Picea engelmannii Parry
Pinus flexilis James
Pseudotsuga menziesii (Mirb.) Franco
Quercus gambelii Nutt.

GRASSES

Agropyron trachycaulum (Link) Malte
A. riparium Scribn. and Smith
Bromus inermis Leyss.
B. polyanthus Scribn.
B. porteri (Coult.) Nash
Hordeum depressum (Scribn. and Smith) Rydb.
Poa nervosa (Hook.) Vasey
P. nevadensis Vasey
P. sandbergii Vasey

FORBS

Achillea millifolium L.
Aquilegia caerulea James
A. flavescens S. Wats.
Artemisia ludoviciana Nutt.
Chenopodium album L.
Descurainia pinnata (Walt.) Britton
Gilia aggregata (Pursh) Spreng.
Heracleum lanatum Michx.
Osmorhiza occidentalis (Nutt.) Torr.
Penstemon rydbergii A. Nels.
P. subglaber Rydb.
Pontentilla gracilis Dougl.
Rumex mexicanus Meisn.
Senecio serra Hook.

Seven species with 12 to 92 percent positive tetrazolium reaction germinated at two or more of the alternating temperatures or at 2°C (table 1). There was no additional germination of stratified seeds after transfer to 20°/10°C. Germination of *Agoseris glauca* and *Melica bulbosa* was appreciable throughout the range of test temperatures. Germination in the other five species was minimal, with temperature preferences at the low end for *Stipa* spp., at the intermediate level for *Agastache urticifolia* and *Moldavica parviflora*, and without a trend in *Polemonium foliosissimum* (table 1).

Table 1.--*Germination percentages of old seed at three temperature alternations and at 2°C*

Species	Temperature alternation (8/16 h)			
	25°/15°C	20°/10°C	15°/5°C	2°C
Agastache urticifolia (Benth.) Kuntze	2	5	3	1
Agoseris glauca (Pursh) Raf.	12	47	44	36
Melica bulbosa Geyer	41	34	58	37
Moldavica parviflora (Nutt.) Britton	0	6	2	0
Stipa columbiana Macour	1	7	12	15
S. lettermani Vasey	0	0	4	1
Polemonium foliosissimum A. Gray	4	0	1	0

Germination of new seed of *S. columbiana* and *S. lettermani* from Ephraim Canyon gave maximum percentages of 77 and 64, respectively, at 15°/5°C. Similarly, *A. urticifolia* yielded 87 percent at 20°/10°C. In new collections from other high elevation sites in Utah, *A. glauca* germinated 24 percent at temperature alternations of 17°/12°C, and to a total of 77 percent during 8-week stratification and after transfer to 22°/17°C (McDonough 1970). *M. bulbosa* germinated 41 percent during and after stratification, but not at higher temperatures, while *P. foliosissimum* germinated 61 percent at 22°/17°C (McDonough 1969). New collections were not available for *M. parviflora*.

Griswald (1936) reported results on five of the species listed in table 1. Tests were carried out mostly at uncontrolled temperatures in the laboratory or greenhouse. Maximum germination percentages were *M. bulbosa* (94), *M. parviflora* (4), *P. foliosissimum* (30), *S. columbiana* (84), and *S. lettermani* (98). The low germination percentage for *M. parviflora* probably resulted from a dormancy that was not relieved by any of the treatments applied. Since the old seeds of *M. parviflora* reacted 68 percent positive in the tetrazolium test, it is possible that more were germinable but had a dormancy not relieved by any of the test conditions.

The comparisons of germinability in old and recent collections of the seeds, and in initial and 40+ year determinations in seeds of the same collections, show the expected results of generally reduced germination in the old seeds. Exceptions were *A. glauca*, where the range of temperature conditions for germination of the old seeds was broader, and *M. bulbosa*, where a stratification requirement may have been eliminated by the aging. However, varying site factors or ecotypic differentiation could be influential here.

Attempts to increase germinability in the old seeds listed in table 1 by application of biochemicals failed. Germination percentages either did not differ significantly or the treatment reduced germination below the water controls.

Seedling survival generally was good, but only *A. glauca* seedlings developed to seed-set (table 2). The grasses and *M. parviflora* did not progress beyond the juvenile stage during the first growth period, and the plants died during or following the low temperature treatment. *A. glauca* set seed during the first growth period; the other forbs required stratification for flowering (*A. urticifolia*) and for stem elongation without flowering (*P. foliosissimum*) during the second growth period.

Table 2.--*Survival and extent of growth of seedlings derived from old seed*

Species	Seedlings			Extent of growth and development
	Planted	Survived		
	No.		*Percent*	
Agastache urticifolia	22	13	59	Flowering, no seed
Agoseris glauca	127	86	68	Seed-set
Melica bulbosa	149	38	25	3-5 leaf stage
Moldavica parviflora	8	3	37	8-14 leaf stage (rosette)
Stipa columbiana	35	7	20	1-3 leaf stage
S. lettermani	5	0	0	1-3 leaf stage
Polemonium foliosissimum	10	4	40	Stem elongation